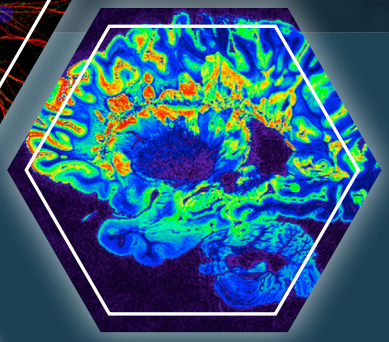
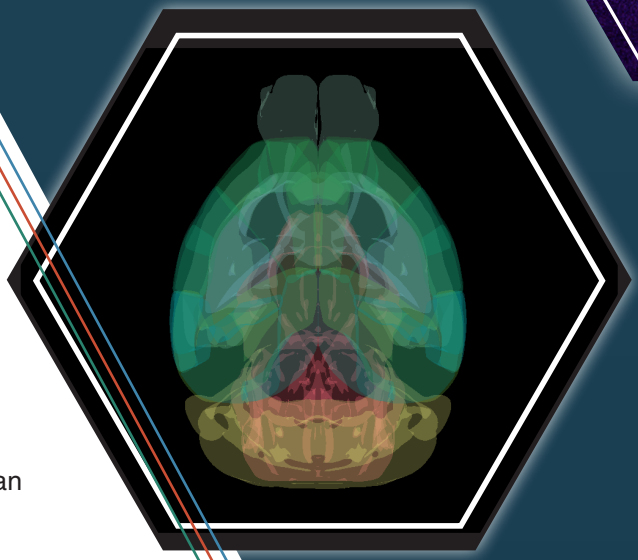
AUGUST 2023

Toolmakers Newsletter





ISSUE 11

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BRAIN INITIATIVE ALLIANCE

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Welcome Back to the Toolmakers Newsletter!

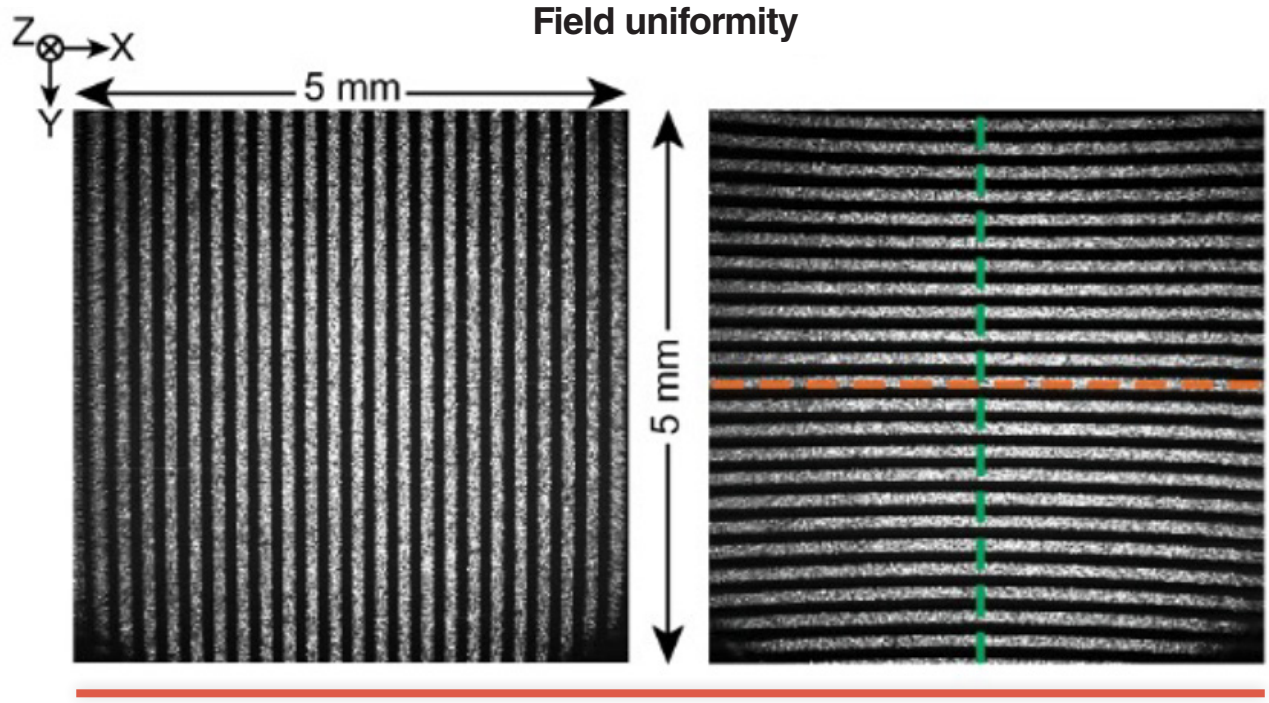

Image: Diesel2p XY images of a calibrated, structured fluorescent sample with a periodic line pattern (5 lines per millimeter) in two orientations acquired under the full scan range of the system. Each image shows 25 lines on the top edge (left image) and on the left edge (right image), receptively, verifying a 5×5 mm field-of-view. Credit: Yu et al., 2023, *Nature Communications*.

Welcome to the third *Brain Research Through Advancing Innovative Neurotechnologies*® (BRAIN) Initiative Alliance Toolmakers Newsletter of 2023!

In this issue, we are proud to showcase a few recent advancements in neurotechnology and demonstrate how they are helping the researchers who developed them and the greater scientific community: Dual Independent Enhanced Scan Engines for Large field-of-view Two-Photon imaging (Diesel2p) by Dr. Spencer Smith; Neuroscience AntiBody Open Resource (NABOR) by Dr. Melina Fan; Pinpoint by Dr. Daniel Birman; and Postsynaptic Accelerated Sensor of Action Potentials (postASAP) by Dr. Rafael Yuste. Let's investigate more about these tools and learn from the scientists behind them!

On the front cover: Top Right Hexagon: An image still from a video of the *C. elegans* brain, including every nerve and muscle fiber, being reconstructed by serial-section electron microscopy. Credit: Daniel Witvliet, University of Toronto and Harvard University, 2020. **Top Central Hexagon:** Four-week-old rat cortical neurons labeled for dendrites (red), axons (green), and nuclei (blue). Credit: Karthik Krishnamurthy, Davide Trotti, Piera Pasinelli, Thomas Jefferson University, 2020. **Bottom Right Hexagon:** A pseudo-colored image of high-resolution gradient-echo MRI scan of a fixed cerebral hemisphere from a person with multiple sclerosis. Credit: Govind Bhagavatheeshwaran, Daniel Reich, National Institute of Neurological Disorders and Stroke, National Institutes of Health, 2016. **Bottom Central Hexagon:** Top-down view of the mouse brain rendered by Pinpoint software as seen on a web browser. Credit: https://data.virtualbrainlab.org/Pinpoint/.

Dual Independent Enhanced Scan Engines for Large field-of-view Two-Photon imaging (Diesel2p) – Dr. Spencer LaVere Smith

The Diesel2p system is a two-photon microscope characterized by its large ~25mm^2 field-of-view with two independent scan engine arms. With these features, the system can perform simultaneous dual-region imaging of neuronal activity throughout multiple cortical areas. To enable the Diesel2p's large field-of-view, it was custom-designed, and the entire system was built from scratch. The optics deliver high resolution (submicron lateral, and about seven microns axial) across a flat imaging plane of focus. Additionally, because each arm operates on its own, image parameters can be individually optimized to scan different brain regions at the same time and deliver multiphoton optogenetic stimulation patterns.

Dr. Spencer Smith's lab at the University of California, Santa Barbara, has been using the Diesel2p to study neuronal activity across multiple brain regions simultaneously *in vivo* in behaving mice. The flexible, customizable features of the Diesel2p system enable and facilitate complex experiments. For example, the system's objective has a long 8 mm working distance in air, plus an adjustable correction collar, ensuring high resolution imaging across a range of preparations without requiring the maintenance of a water interface. This design feature also makes it more compatible with probe instruments and easier to adjust to diverse or challenging animal preparations.

The two arms of the Diesel2p system are essential for imaging during multiphoton optogenetic stimulation. "We are studying neuronal activity during complex sensory processing and behavior, in primary visual cortex and an array of higher visual cortical areas and other brain regions," says Dr. Smith. "The Diesel2p lets us survey multiple areas simultaneously and compare across areas even when there is no uniform trial structure." When designing the Diesel2p, extra configurations were made to the scan engine to ensure it could provide maximum optical access—it needed to be flexible, allowing for modification as needed, and it needed to easily integrate multiphoton optogenetic stimulation and adaptive optics.

Labs across the world are currently using the Diesel2p system in their experiments, with some using the system as designed, and others taking advantage of its flexible nature and making modifications. Dr. Smith's lab is thrilled that so many researchers are using it and tailoring it as necessary for their own brain imaging requirements. The Smith lab continues to work on developing more optics tools for use in the larger neuroscience community, for example the ultra-long working distance, multiphoton-optimized Cousa objective. The Diesel2p system is open source, and you can view its design in *Nature Communications* (2021). Two independent companies, INSS and Cosys, have developed systems based on the Diesel2p platform.

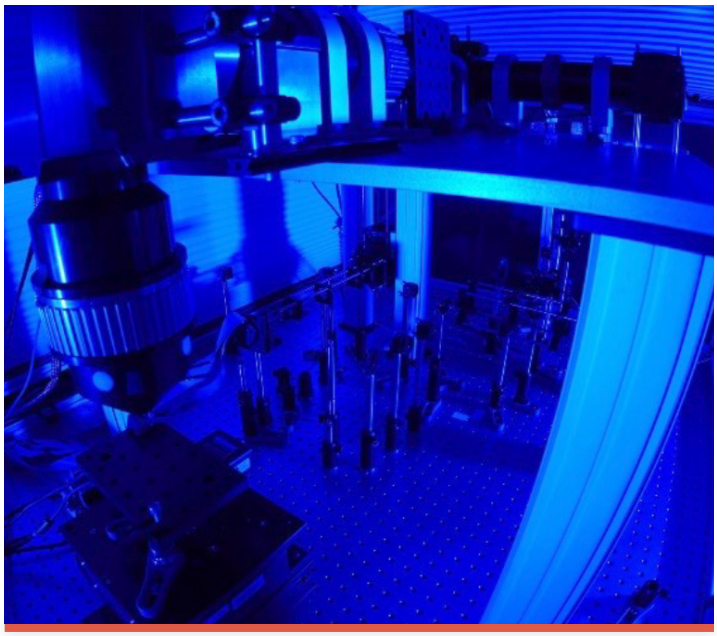

Image: The Diesel2p system. Credit: Spencer Smith / SLAB, 2023.

> "In the Diesel2p, the two scan arms are combined using polarization, not wavelength, so both scan engine arms can use the same wavelengths."
>
> — *Dr. Spencer Smith*

Neuroscience AntiBody Open Resource (NABOR) – Dr. Melina Fan

NABOR is an open source collection of antibodies and affinity reagent resources for distribution to the larger neuroscience community. It is a collaboration between Addgene, a global nonprofit repository for DNA-based research reagents, and the Trimmer Lab at the University of California, Davis. Through NABOR, Addgene hopes to expand access to recombinant antibodies and associated information—which is why they publish online protocols, antibody guides, blog posts, and informative videos to help support other scientists and drive scientific innovation and success. As of summer 2023, NABOR contains over 150 antibodies and over 250 NeuroMab generated plasmids.

In addition to the antibodies themselves, the NABOR team takes pride in making the validation data easily accessible—they currently have over 635 validation reports for the available antibodies in the collection. The NABOR team also performs quality control and application testing on their antibodies to help other scientists know which reagents might be most helpful for their experiments. Finally, NABOR shares the amino acid sequences of the antibodies when available, which improves reproducibility and allows scientists to improve upon the tools.

NABOR was first launched in March 2022 and has quickly grown. In fall 2023, the NABOR team is launching anti-integrin antibodies via a new partnership with the Institute for Protein Innovation (IPI), with more antibody tools expected to come. The team will also be increasing NABOR's neuroscience function soon by assessing antibody applicability in tissue clearing methods, building isotype conversion capabilities, and building hybridoma sequencing. Dr. Kwangyun Chung, Dr. Heng Zhu, and CDI Labs are working with Addgene to provide hybridomas for antibody development as part of the NIH BRAIN Cell Atlas Network (BICAN). "We hope that by creating a hybridoma sequencing platform, we will help members of the community interested in long-term preservation and sharing of monoclonal antibodies developed in their labs," says Dr. Meghan Rego, Director of Antibodies at Addgene.

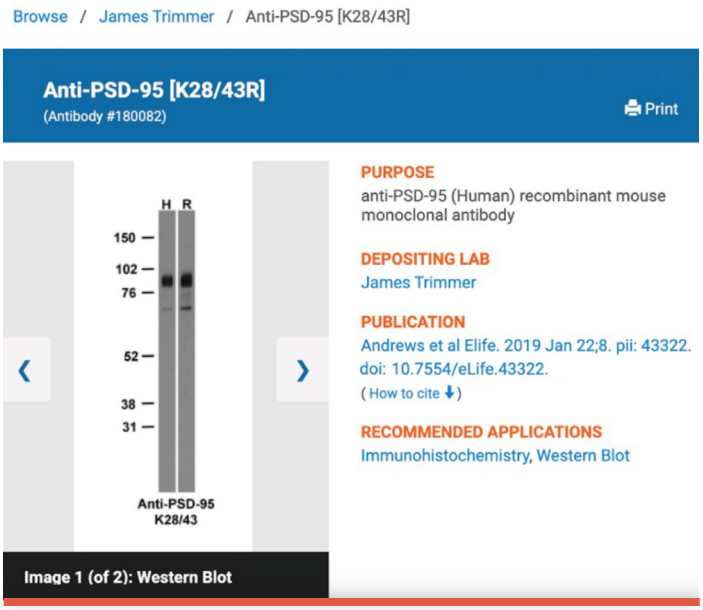

Image: Plasmids and ready-to-use recombinant antibodies, such as this Anti-PSD-95 antibody, is shared by researchers, including the NeuroMab collection. Credit: Addgene, 2023.

NABOR is a constantly growing collection made possible through scientific engagement. If you would like to know more about sharing through NABOR or are interested in contributing to the collection, contact Addgene at help@addgene.org.

> "We are excited to continue working with the neuroscience community and are always looking for collaborators who are making new tools, have new technologies to improve current recombinant antibody tools, or who would like to participate in antibody validation."
>
> *— Dr. Melina Fan*

Pinpoint – Dr. Daniel Birman

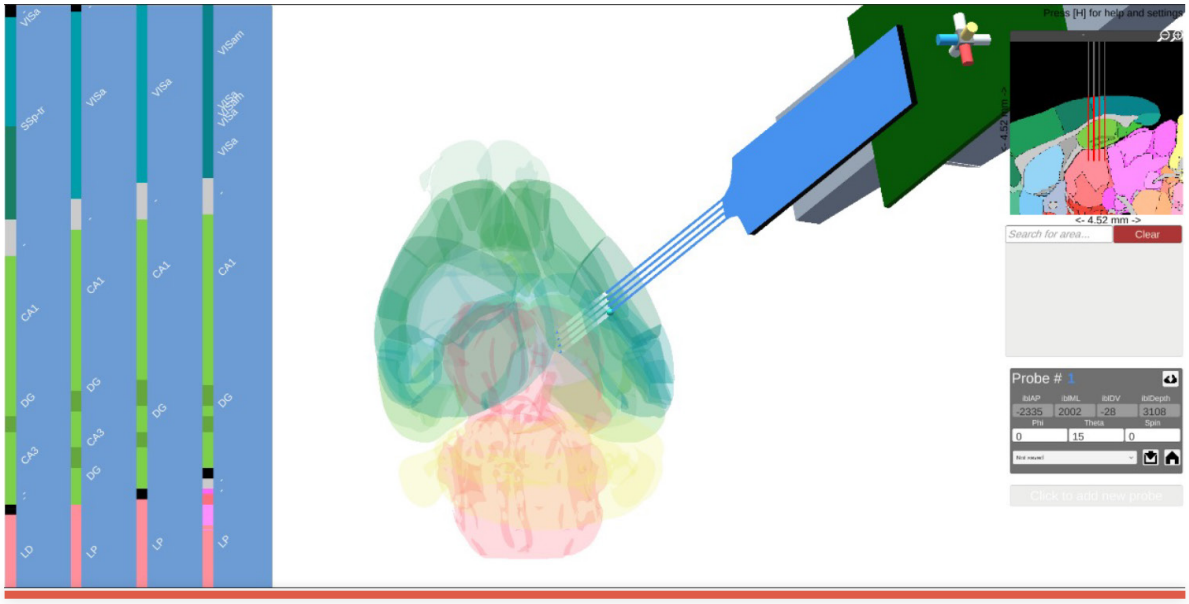

Image: Multi-probe planning for Neuropixels using interactive 3D visualization in a web browser. Credit: Virtual Brain Lab, 2023.

At the University of Washington, Dr. Daniel Birman is leading the Virtual Brain Lab, a hub for interactive tools for neuroscience applications. One of the projects developed by the Lab is Pinpoint, a piece of 3D visualization software that helps researchers virtually plan electrophysiology experiments and recordings. This type of software is especially useful when planning multi-probe surgical procedures and craniotomies.

> "Pinpoint has really revealed to our team and to the International Brain Laboratory how useful it is to have interactive tools that let you explore your data as you plan experiments and analyze results."
>
> — *Dr. Daniel Birman*

Pinpoint's features support the entire multi-probe insertion procedure planning process. Users can pick which brain area they would like to target, select probe or rig settings, examine and alter probe trajectories, and check for any roadblocks to probe positioning to ensure probes are compatible with other surgical implant parts.

In addition to the virtual interactive component, Pinpoint is also being used for live recordings in mice—it can mimic the positioning of real probes, helping scientists place probes while being able to visualize where they are. If a researcher wants to deviate from their experimental plan and make changes to probe placement, they can use Pinpoint to see exactly how those changes will affect other probe positioning. To extend on this capability, Pinpoint is on the brink of automation—Dr. Birman and his team are in the process of adding a software-to-hardware interface so that Pinpoint can read and interface with micro-manipulators used during experiments. This way, Pinpoint will be able to give a live report of where probes are placed within the brain while a researcher is inserting them.

The team hopes to eventually automate the insertion process as much as possible to help make experiments quicker and minimize the amount of manual work needed. "If it takes an expert researcher 15 minutes to insert a single probe, and 10 extra minutes for each additional probe, a 6-probe insertion takes over an hour," says Dr. Birman. "With our new "Copilot" feature we keep the total time at about 15 minutes by doing all of the insertions at the same time."

Pinpoint is free and available for anyone to use here. The success of this software has led Dr. Birman's lab to build similar software for data analysis and consider how related tools or capabilities could help visualize and explore their data better.

Postsynaptic Accelerated Sensor of Action Potentials (postASAP) – Dr. Rafael Yuste

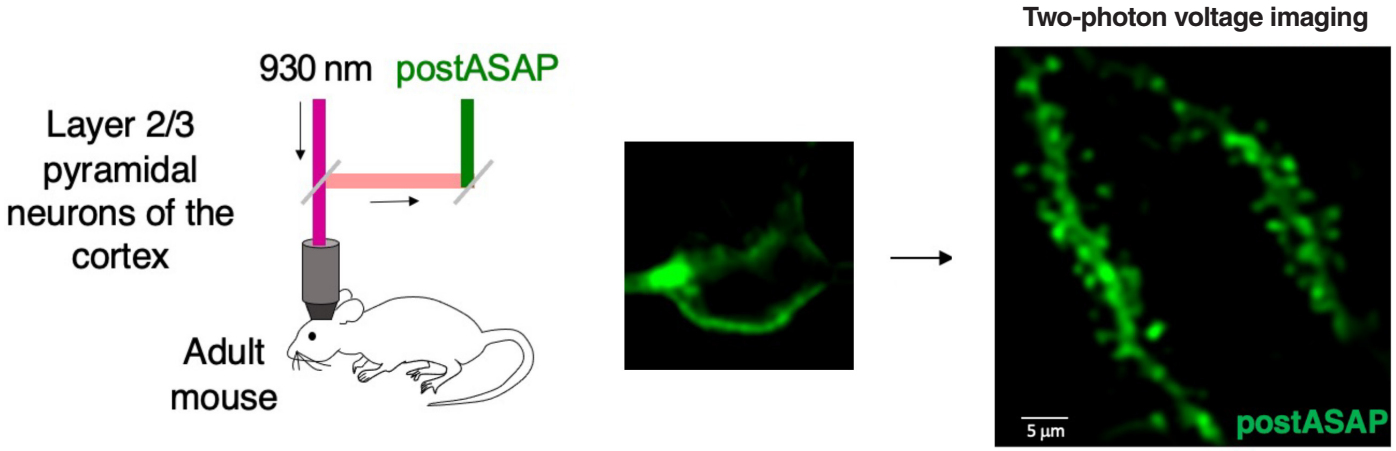

Image: Experimental setup and images showing the expression of postASAP in a pyramidal neuron of the somatosensory cortex in mice. Expression can be observed in the cell body, as well as in the dendrites and spines of the same cell. Credit: Cornejo et al., 2022, *Science*.

postASAP is a genetically encoded voltage indicator (GEVI) that measures electrical function and activity of tiny, individual synapses in the mouse brain with great sensitivity. The project began when Dr. Rafael Yuste's lab at Columbia University sought to develop a GEVI that was less invasive than previous techniques while measuring dendritic voltage dynamics and spine voltages *in vivo*. postASAP is extremely compatible with two-photon microscopy, allowing the lab to study neural activity in living rodents, measure voltage dynamics, and to study optogenetic activation.

> "We are confident that further applications in other mouse brain regions or animal models could greatly benefit the neuroscience community and address relevant questions in modern neuroscience."
>
> — *Dr. Victor Hugo Cornejo*

With the help of postASAP, the team at Dr. Yuste's lab images voltages within dendritic spines to learn more about synaptic dysfunction that causes neurological conditions like schizophrenia or autism spectrum disorders, and how to regulate voltage compartments. The phenomenon of voltage compartmentalization in dendritic spines is being continuously studied by neuroscientists investigating if neurons can isolate electrical signals in compartments. "For years, only theoretical and computational models of voltage compartmentalization have supported this isolation," says Dr. Victor Hugo Cornejo, a postdoctoral scientist in Dr. Yuste' lab, "but in recent years, we have been able to demonstrate it in cultured cells or brain slices." In 2022, the lab published a paper in *Science* that supports their hypothesis on voltage compartmentalization and details their protocols and evidence in living animals.

The team at Columbia University continues to expand on their work from the last few years, with the main goal of improving postASAP's measurements. In fact, a newer, enhanced version of postASAP could be on the horizon with improved features such as a higher signal-to-noise ratio in individual spines and better temporal profiling for speedy synaptic potentials. All these improvements will come with increased precision in electrical activity measurements and will continue to be complemented by two-photon microscopy.

The plasmids that Dr. Yuste's lab uses with postASAP for voltage sensor targeting are available through Addgene, discussed above in this newsletter.

Excited by the potential of the tools in this issue?! Stay tuned for our next issue and explore more products of BRAIN Initiative discoveries in our Toolmakers' Resources page!